RESISTANCE TO *PUCCINIA GRAMINIS TRITICI* IN KHAPSTEIN, A *VULGARE* DERIVATIVE of KHAPLI EMMER.

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Synopsis.

The results of studies with Khapstein, a stem rust resistant *vulgare* derivative of Khapli emmer, are reported. The mode of inheritance was studied and the relationship of this resistance to that of Marquillo, Thatcher, Hochzucht, Kenya 744 and Kenya 117A was found. Khapstein appears to be a useful source of resistance to the Australian stem rusts since it crosses readily with common wheats.

INTRODUCTION.

The availability of an effective source of resistance to *Puccinia graminis tritici* is basic to breeding programmes aimed at the development of rust resistant varieties. It is now well recognized that the extreme variability shown by this pathogen can be combated most effectively by varieties whose genes for resistance have come from diverse and unrelated sources. Khapli emmer provides genes which are unlike those used previously in breeding stem rust resistant bread wheats and, moreover, these genes appear to be effective against all but a few of the hundreds of races that occur throughout the world.

Although the resistance of Khapli has been known for many years, there have been difficulties in transferring this character to *vulgare* wheats. As a result of studies made over a period of twenty years at the University of Sydney, Steinwedel has been found useful for successful crosses with Khapli (Waterhouse, 1930, 1933, 1952). One selection from Waterhouse's crosses, a Steinwedel × Khapli cross, has been provisionally registered as Khapstein. The selection was made mainly on the basis of resistance to stem rust. Hence it is unlikely to be a commercial success, since it has several undesirable agronomic characters. In New South Wales, under severe rust epidemics involving all known local races and biotypes, Khapstein has shown a valuable resistance. From glasshouse tests seedlings and adult plants were found to be resistant to all stem rusts occurring in Australia. As it is used extensively in breeding, the present investigations were undertaken to determine the mode of inheritance of stem rust resistance. The crosses were planned so that a study could also be made of any relationship that might exist between the genes of Khapstein and those already identified by the writers (1954, 1955) in Kenya 744*, Kenya 117A 1347, Marquillo 724, Thatcher 1201, and Hochzucht 1227.

REVIEW OF LITERATURE.

Hayes and Stakman (1922) obtained unsatisfactory results from their attempts to cross Khapli with Marquis. Hynes (1926) reported a successful cross between Federation and Khapli and he interpreted the results on the basis of multiple factors for stem rust resistance. Similar conclusions were drawn by Aamodt (1927) as a result of crosses between Khapli and a *durum* wheat. Waterhouse (1930) in Khapli × *durum* crosses obtained results which indicated that the resistance of Khapli was dependent on two dominant factors. In Khapli × *vulgare* crosses Waterhouse (1930, 1933, 1952) used more than 300 varieties and found that the compatibility with Khapli depended on the variety. Steinwedel was among a group of varieties that gave F_1 plants from which fertile lines could ultimately be selected. One of his lines having the stem rust resistance of Khapli and previously referred to as K.D. 1451 (Watson and Singh, 1952) has now been named Khapstein.

* Varieties carry the Sydney University Accession Number.

Materials and Methods.

The present investigations with Khapstein are a continuation of the inheritance studies on Marquillo, Thatcher, Hochzucht, Kenya 744 and Kenya 117A reported earlier (Athwal and Watson, 1954, 1955). Various generations of material of the following crosses, all of which involve Khapstein as one of the parents, were used: Federation 107 × Khapstein 1451; Khapstein 1451 × Hofed 1200; Marquillo 724 × Khapstein 1451; Thatcher 1201 × Khapstein 1451; Hochzucht 1227 × Khapstein 1451; Kenya 744 × Khapstein 1451; Kenya 117A × Khapstein 1451.

The characters of seven of these parents have been previously described by the writers. The spike of Khapstein (Steinwedel × Khapli) is awnleted, clavate and compact; the glumes are glabrous and brown and the kernels white and long. It is resistant to all available biotypes of races 21, 126 and 222 occurring in Australia.

All results were obtained in the glasshouse at moderate temperatures (67–72°F.), using races 21, 126 and 222 of stem rust. Race 21 is from India and is quite unlike race 21Anz. 1 occurring locally in that Marquillo is susceptible to the latter (Watson, 1955). One biotype only of race 126 was used, viz. 126Anz. 1, but data were obtained from two biotypes of race 222, viz. 222AB (Anz. 2) and 222BB (Anz. 3). Seedlings of Hofed and Federation are susceptible to these races, and each was crossed with Khapstein to determine the mode of inheritance.

The initial tests were done with race 222AB. F_4 lines whose reaction to this race was known were then tested with races 21, 126 and 222BB and thus a comparison of the breeding behaviour of these lines to the different races was determined.

The crosses of Khapstein with the resistant varieties Marquillo, Thatcher, Hochzucht, Kenya 744 and Kenya 117A were tested with races 222AB and 21 to determine what relationship, if any, was shown in the resistances of the different varieties. It was found that there were gradations of reaction between resistance and susceptiblity in the F_2 generation of these crosses and difficulty was encountered in classifying the individual plants. Plants with an intermediate type of reaction occurred in the F_2 generation but F_3 breeding behaviour showed that these plants resembled closely other plants classified as semi-resistant. Definite indications on the mode of inheritance in such cases could therefore only be obtained from a study of the F_3 generation. In crosses between two resistant varieties the main emphasis was placed on the isolation of homozygous susceptible F_3 lines.

Random samples of F_2, F_3 and F_4 populations of crosses were employed in all cases. The following classes were used to record the seedling reactions:

Immune	(I)	0
Highly Resistant	(R+)	0;
Resistant	(R)	0,; and 1 or 0,; and $2^{=}$
Moderately Resistant	(R–)	0,; and 2, 2^{-}, $\times^{-}$ or $3^{=c}$
Semi-resistant	(SR)	2, 2^{+}, × or 3^{-c}
Intermediate	(Int.)	3^{c} or $\times^{+}$.
Susceptible	(S)	3, 4
Segregating	(Seg.)	F_3 and F_4 lines segregating for resistant and susceptible individuals.

If the hybrid lines showed a gradation of reactions they were defined by an appropriate range as, for example, R+ to SR, R– to SR, etc.

Experimental Results.

The reactions of the parents of the crosses studied here to races 126Anz. 1, 126Anz. 2, 222AB (Anz.2), 222BB (Anz. 3) and 21 are given in Table 1. These are average reactions at 67–72°F. In the table the reaction of Khapli is also given so that a ready comparison with Khapstein can be made. Their reactions to race 15 have been included to show certain differences between them.

Inheritance Studies with Race 222AB.—Six F_1 seedlings of a cross between Federation and Khapstein gave a semi-resistant reaction. Seedlings of Khapstein under the same conditions were moderately resistant. It was concluded that the reaction of

Khapstein was incompletely dominant. The reactions of F_2 plants of crosses of Khapstein with Federation and Hofed are given in Table 2.

The F_2 data of the cross Federation × Khapstein in Table 2 do not agree with a 3:1 ratio ($P < 0{\cdot}05$). If the classes showing the intermediate and the resistant types of reaction are grouped, the P value for agreement of those data with a 13:3 ratio lies between 0·05 and 0·10. When these same classes are combined in the Khapstein × Hofed cross the P value for the 13:3 ratio lies betwen 0·20 and 0·30.

TABLE 1.

Reactions of the Parental Varieties and Khapli to Several Races of Stem Rust.

Variety.	Race No.					
	126 Anz. 1	126 Anz. 2.	222AB (Anz. 2).	222BB (Anz. 3).	21	15
Federation ..	S	S	S	S	S	—
Hofed	S	S	S	S	S	—
Khapstein ..	R−	R−	R−	R−	R−	SR & Int.
Khapli ..	R	R	R	R	R	R
Marquillo ..	R+	R+	R+	R+	R & R−	—
Thatcher ..	R+	R+	R+	R+	I	—
Hochzucht ..	R+	R+	R+	R+	I	—
Kenya 744 ..	R−	R−	R−	R−	R−	—
Kenya 117A ..	R−	R−	R−	R−	R−	—

Some of the F_2 plants of the cross Federation × Khapstein classified for their reaction to race 222AB were transplanted in the field and the breeding behaviour of the F_3 seedling progeny was studied using the same race of rust. Table 3 summarizes the relationship between the reaction in F_2 and F_3.

Table 3 shows that 73 resistant F_2 plants gave either homozygous resistant or segregating F_3 progenies, except one which gave a susceptible progeny. In the F_2 generation it was difficult to separate semi-resistant and susceptible reactions from the intermediate ones, but most of the plants classified as intermediate gave either semi-resistant or segregating progenies and their breeding behaviour justifies their inclusion in the resistant class for the interpretation of F_2 data. The unexpected

TABLE 2.

Reactions of F_2 Plants of the Crosses Federation × Khapstein and Khapstein × Hofed to Race 222AB.

	Reactions.			
	Resistant R− and SR.	Intermediate.	Susceptible.	Total.
Federation × Khapstein ..	804	80	233	1117
Khapstein × Hofed	468	39	131	638

behaviour of one susceptible F_2 plant which gave resistant progeny and one semi-resistant plant which gave susceptible progeny is probably due to a mistake either during the F_2 classification or during transplanting.

It may also be seen from Table 3 that approximately two-thirds of the susceptible F_2 plants gave segregating progenies; the remainder bred true for susceptibility. Among these latter families, however, an occasional plant was sometimes found having a degree of resistance. Lines showing such plants have been classified as homozygous susceptible when interpreting the data. It is not uncommon to find aberrations in the segregation of crosses involving *vulgare* parents whose resistance has been derived from 14-chromosome types.

The results on the inheritance of resistance can be explained by assuming that two factors differentiate the resistance of Khapstein from the susceptibility of

Federation. One of these factors is dominant, the other recessive, and when either is present singly partial resistance is shown. Of the 115 F_3 lines in Table 3, 42 possessed a certain amount of resistance but most of these did not equal the resistance of Khapstein. The numbers of resistant (42), segregating (62) and susceptible lines (11) agree with a 7:8:1 ratio with a P value of 0·10 to 0·20.

TABLE 3.

The Reactions to Race 222AB of the Progeny of 115 Tested F_2 Plants of the Cross Federation × Khapstein.

F_2 Reactions.	F_3 Reactions.					
	Resistant.		Segregating.		Susceptible.	Total.
	(R– to SR.)	(SR.)	(R–, SR and S.)	(SR and S.)		
Resistant :						
R– ..	13	10	8	—	—	31
SR ..	8	8	18	7	1	42
Intermediate ..	—	2	7	7	2	18
Susceptible ..	1	—	11	4	8	24
Total ..	22	20	44	18	11	115

More comprehensive data on a sample of 232 F_3 lines of the cross Federation × Khapstein is contained in Table 4. In the segregating lines, the numbers of R–, SR and S plants were counted and these lines can be classified as R– and SR > S (preponderance of moderately resistant and semi-resistant plants), SR > S (preponderance of semi-resistant plants) and S > SR (mostly susceptible plants). If

TABLE 4.

The Probable F_2 Genotypes and Their Breeding Behaviour in F_3 of Plants of the Cross Federation × Khapstein Tested with Race 222AB.

F_3 Breeding Behaviour.	Probable F_2 Genotype.	Numbers of Lines.	
		Observed.	Expected.
Resistant :			
R–	Kph_1 Kph_1 kph_2 kph_2	20 } 113	14·5 } 101·5
SR or R– to SR ..	Kph_1 Kph_1 Kph_2 kph_2 Kph_1 kph_1 kph_2 kph_2 Kph_1 Kph_1 Kph_2 Kph_2 kph_1 kph_1 kph_2 kph_2	93	87·0
Segregating :			
R– and SR>S } SR>S } ..	Kph_1 kph_1 Kph_2 kph_2 } Kph_1 kph_1 Kph_2 Kph_2 }	77 } 105	87·0 } 116·0
S>SR	kph_1 kph_1 Kph_2 kph_2	28	29·0
Susceptible :			
S	kph_1 kph_1 Kph_2 Kph_2	14	14·5
Total	.. ..	232	232·0

Kph_1 and kph_2 are the dominant and recessive factors respectively in Khapstein the probable genotypes of the F_2 plants and their breeding behaviour in F_3 to race 222AB are given in Table 4.

The numbers of resistant, segregating and susceptible lines in Table 4 agree with a 7:8:1 ratio, the P value being approximately 0·30. While these reactions were being recorded a special note was taken of those lines as resistant as Khapstein and the results show that only 20 of the 113 lines in the resistant category equalled the moderate resistance (R–) of Khapstein. There was a preponderance of susceptible plants in 28 of the 105 segregating lines. It appears from this that the two factors for resistance on Khapstein are additive in effect and only when present together can they produce a reaction equal to that of the parent. As one of these factors behaves as a recessive and the other as a dominant, the observed frequencies of five categories of reaction in Table 4 are also expected to agree with a 1:6:6:2:1 ratio. The P value for agreement with this ratio lies between 0·30 and 0·50.

Relationship of the Resistance to Various Races.—A random sample of 127 F_4 lines of the cross Federation × Khapstein, whose reaction to race 222AB was known, were tested with races 21, 126Anz. 1 and 222BB. The reaction to these latter three races was identical and similar to that given to race 222AB. Of the 127 lines, 50 were moderately resistant or semi-resistant, 72 segregated for different grades of resistance and susceptibility, and five were homozygous susceptible. It was concluded, therefore, that the same two genes in Khapstein control resistance to all four races of rust.

TABLE 5.

Reactions of F_1 and F_2 Plants to Races 21 and 222AB of Crosses between Khapstein and Other Resistant Varieties.

	Gen.	Race.	Reactions.					
			Resistant.			Int.	Sus.	Total.
			I.	R+ & R.	R– & SR.			
Marquillo × Khapstein	F_1	222AB	—	—	4	—	—	4
Marquillo × Khapstein	F_1	21	—	—	4	—	—	4
Marquillo × Khapstein	F_2	222AB	—	777*	—	20	9	806
Thatcher × Khapstein	F_1	222AB	—	—	3	—	—	3
Thatcher × Khapstein	F_1	21	5	—	—	—	—	5
Thatcher × Khapstein	F_2	222AB	—	160	569	22	11	762
Thatcher × Khapstein	F_2	21	164	—	40	8	3	215
Hochzucht × Khapstein ..	F_1	222AB	—	—	4	—	—	4
Hochzucht × Khapstein ..	F_1	21	4	—	—	—	—	4
Hochzucht × Khapstein ..	F_2	222AB	—	259	829	26	16	1130
Hochzucht × Khapstein ..	F_2	21	513	—	161	6	10	690
Khapstein × Kenya 744	F_1	222AB	—	—	5	—	—	5
Khapstein × Kenya 744	F_2	222AB	—	—	801	18	24	843
Khapstein × Kenya 117A ..	F_1	222AB	—	—	9	—	—	9
Khapstein × Kenya 117A ..	F_2	222AB	—	—	1140	40	34	1214

* Plants showing different grades of resistance have been grouped together.

Relation of Khapstein to the Other Resistant Varieties.—In order to investigate this relationship the following crosses were studied: Marquillo × Khapstein; Thatcher × Khapstein; Hochzucht × Khapstein; Kenya 744 × Khapstein; and Kenya 117A × Khapstein.

Previous work by Athwal and Watson (1954, 1955) had shown that in Marquillo, Thatcher and Hochzucht there is a group of genes for resistance to race 222AB. Kenya 744 and Kenya 117A have a single factor for resistance to this same race. Thatcher and Hochzucht have as well a factor for immunity to race 21. The crosses were so planned that the relationship of Khapstein to each of these resistances could be studied. In view of the genetic similarity between some of the varieties all the crosses listed above are not necessary; nevertheless the results obtained are given in Tables 5 and 6.

It will be seen from Tables 5 and 6 that the three crosses of Khapstein with Marquillo, Thatcher and Hochzucht have behaved similarly in that susceptible segregates were obtained in the F_2 and F_3 generations. This similarity would be expected from the results obtained in previous studies (Athwal and Watson, 1955). In view of the complexity of inheritance in Marquillo, Thatcher and Hochzucht it is not possible to fit the data to any genetic ratio. However, it is evident that the genes for resistance present in these varieties are not allelic to those in Khapstein. Furthermore, the results with race 21 show that the immunity factor in Thatcher and Hochzucht is epistatic to, and non-allelic with, either of the two factors in Khapstein.

In the crosses of Khapstein with Kenya 744 and Kenya 117A three factors are segregating for reaction to race 222AB. Khapstein contributes two factors, one dominant and the other recessive, and a single factor comes from each of the Kenya varieties. The two crosses show the same type of F_2 and F_3 segregations, although there is a slight difference in the percentage of segregating lines (Table 6). No explanation can be given for this. The F_2 data on 843 plants of the cross involving

TABLE 6.

Reactions of F_3 Lines to Races 21 and 222AB of Crosses between Khapstein and Other Resistant Varieties.

Cross.	Race.	Reactions.							
		Resistant.				Segreg.†			
			R+ to R.	R− to SR.	R to SR.	a.	b.	Sus.	Total.
Marquillo × Khapstein ..	222AB	—	17	23	51	20	14	1	126
Thatcher × Khapstein ..	222AB	—	16	83	133	25	18	—	275
Thatcher × Khapstein ..	21	49	—	45	101	— 27	‡ —	1	223
Hochzucht × Khapstein	222AB	—	22	90	164	38	23	2	339
Kenya 744 × Khapstein..	222AB	—	—	298	—	89	46	4	437
Kenya 117A × Khapstein	222AB	—	—	137	—	35	5	1	178

† Lines segregating as does an F_2 of the cross are recorded under " a " ; those which segregate with approximately 25 per cent. or more susceptible are recorded under " b ".

‡ Lines under " a " and " b " have been grouped.

Kenya 744 and 1214 plants of the Khapstein × Kenya 117A cross (Table 5) show that 24 and 34 plants respectively were susceptible. The results do not agree with the expected ratio of 61:3 ($P < 0{\cdot}05$) and the deficiency of susceptible plants suggests a loose linkage between one of the genes in Khapstein and the gene for resistance in the Kenya varieties. A deficiency of homozygous susceptible F_3 lines (Table 6) further strengthens this possibility.

DISCUSSION.

The inheritance studies with Khapstein show that the resistance of this variety is governed by two independently inherited factors, one of which is dominant, the other recessive. Each of these two factors can produce a partially resistant reaction when present singly and their action appears to be additive. Previous work is conflicting in that Hynes (1926) and Aamodt (1927) attributed the resistance of Khapli to multiple factors, while Waterhouse (1930) concluded from results with race 43 that the resistance was due to two dominant factors.

It is possible that the inheritance of resistance in Khapli is much more complex than that in Khapstein. The latter is not as highly resistant to the Australian rusts as Khapli and it could be concluded that the full complement of genes conditioning rust resistance in the latter have not been transferred to Khapstein. Whereas the differences in the reaction to local rusts shown by those two varieties are not spectacular, a culture of race 15 from India makes evident clear-cut differences between them. Khapli is resistant to this race but Khapstein gives only intermediate and semi-resistant types of reaction. It will not be surprising, therefore, to find in future certain rusts to which Khapstein is susceptible but Khapli resistant. Similarly the mode of inheritance of Khapli resistance can be expected to vary with the race used on the segregating material.

The two factors for resistance present in Khapstein are not allelic with those in Marquillo, Thatcher and Hochzucht. It may be that they are independent of these factors and of the factor present in Kenya 744 and Kenya 117A. However. there appears to be a linkage between one of the factors in Khapstein and the factor which is effective against Australian rusts in the two above-mentioned Kenya varieties. The presence of ill-defined reactions in the F_2 generation and the size of the F_3 populations do not justify the calculation of linkage intensity. If such a linkage is present it will explain the deficiency of susceptible plants in crosses between Khapstein and Marquillo, Thatcher and Hochzucht (Table 1). The results obtained earlier (Athwal and Watson, 1955) have shown conclusively that the factor in Kenya 744 and Kenya 117A is strongly linked with one of the major factors in Marquillo, Thatcher and Hochzucht. Consequently a linkage would be expected between this major gene present in Marquillo and related varieties and one of the two factors in Khapstein.

As far as Australian races of stem rust are concerned, Khapstein appears to be one of the most valuable sources of resistance. It possesses resistance to all these rusts and the genes responsible are present in a genetic background that allows them to be transferred readily to any of the commonly cultivated varieties. So far no difficulties have been encountered using Khapstein as a donor parent in back-cross programmes that are under way with many varieties as recurrent parents. Moreover, the present studies indicate that it should be possible to combine the Khapstein resistance with that of Marquillo and of Kenya 117A. Such combinations would result in resistances with a broader genetic base than is possible with either component singly and this may lead to a more stabilized resistance (Watson and Singh, 1952).

Summary.

Inheritance studies with Khapstein show that the resistance of this variety to Australian stem rust races and to race 21 is dependent upon two independently inherited factors. One of these factors is dominant, the other recessive, and while each is capable of giving partial resistance when present singly it appears that together their effect is additive. Crosses of Khapstein with certain resistant varieties show that the two factors are not allelic with a group of genes for resistance to race 222AB which are present in Marquillo, Thatcher and Hochzucht. Also they are not allelic with a factor for resistance to this same race in Kenya 744 and Kenya 117A nor with the factor for immunity to race 21 present in Thatcher and Hochzucht.

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